

Amendments to the Specification:

Please replace paragraph 0001 on page 1 with the following rewritten paragraph:

This application is a continuation-in-part of U.S.S.N. 10/206,386, filed July 26, 2002, now abandoned, which is a continuation in part of U.S.S.N. 10/013,847, filed October 30, 2001, now U.S. Patent No. 6,695,009, which claims the benefit of U.S. provisional patent application no. 60/244,807, filed October 31, 2000, the entire disclosure of which is hereby incorporated herein by reference in its entirety for all purposes.

Please replace paragraph 0033 on page 9 with the following rewritten paragraph:

Once the substantially static interface between the first and second fluids is established within the first channel 100, the sample material to be concentrated is introduced into the first channel portion 100a. An electric field is then applied through the first and second fluid regions within channel portions 100a and 100b, respectively, e.g., via electrodes 118. The differential electrophoretic velocity of the sample material through the first and second fluids results in concentrated region of the sample material 116 substantially at the interface 114. In the example illustrated in Figures 6A-6D, an electrical field is applied through the three fluid regions by applying a voltage potential from reservoir 630 to reservoir 640. The differential electrophoretic mobility of the sample material through the fluid regions 692 and 694 causes the analyte or sample material to stack at the static interface 690 (b) (or 690 (a)), depending on the direction of the applied electric field ~~which that~~ is applied via reservoirs 630 and 640 and the charge of the sample material or analyte.

Please replace paragraph 0042 on page 12 with the following rewritten paragraph:

The counter current stacking methods of the present invention are schematically illustrated in Figures 4A and 4B. As shown in Figure 4A, the methods employ a channel network ~~400~~ that includes a main concentration channel segment 402. Two channel segments 404 and 406 are provided in fluid communication with the main channel segment at either end of the concentration channel segment 402, and provide bulk fluid flow in a first direction as indicated by the solid arrows (shown in Figure 4B). A second pair of channel segments 408 and 410 is also provided in fluid communication at opposing ends of the concentration channel

segment 402 to provide electrophoretic movement (as shown by the dashed arrows) of charged species in the concentration segment 402 in the direction opposite that of the bulk fluid movement. The combination of bulk fluid flow in one direction and electrophoretic movement in the other direction results in an accumulation of charged species in channel segment 402. Once a desired level of concentration is achieved, one of the two motive forces, e.g., bulk or electrophoretic, is shut off, allowing the other force to predominate, driving the concentrated material out of channel segment 402. The concentrated material is then subjected to additional manipulations, e.g., as described above. The relative levels of electrophoretic or bulk fluid flow are provided using the same systems used in carrying out the static interface concentration aspects of the invention.

Please replace paragraph 0047 beginning on page 13 with the following rewritten paragraph:

Figure 2 illustrates a first exemplary device structure that employs the concentration function of the present invention. In particular, as shown in Figure 2A, the device includes a channel geometry that comprises a simple crossing intersection, e.g., two channels 202 and 204, that cross each other and are in fluid communication at the intersection point or first fluid junction 206. This geometry is optionally described in terms of four channel segments (202a, 202b, 204a and 204b) communicating at a first fluid junction 206. In order to provide a larger area in which sample material could be concentrated, the fluid junction can be readily enlarged, e.g., by offsetting the point at which the cross channel segments (e.g., 202a-204a and 202b-204b) connect with the main channel-204-202. This configuration is illustrated in Figure 2C.

Please replace paragraph 0048 on page 14 with the following rewritten paragraph:

Figure 2B illustrates the channels including the static fluid interface 208, where the ~~region~~-first fluid 210 (indicated by hatching) has a first ionic make-up, e.g., relatively low conductivity, and the ~~region~~-second fluid 212 (indicated by cross-hatching) has a second ionic make-up, e.g., relatively high conductivity. In order to establish the fluid interface, all of the channel segments ~~202a-and, 202b-and, 204a, and 204b,~~ that communicate at the first fluid junction 206 are filled with the first fluid 210. The second fluid 212 is then transported into all

but one of these channel segments by, e.g., pumping the second fluid into the fluid junction 206 through channel segment 204a, and controlling the flow at the junction 206 such that the second fluid only flows into segments 202b and 204b. This yields the channel network shown in Figure 2B with a static fluid interface 208 in the position indicated therein. Alternatively, the entire channel structure can be first filled with the second fluid. The first fluid is then introduced into the sample loading channel segment, e.g., channel segment 202a, and advanced until the fluid interface reaches the desired position.

Please replace paragraph 0056 on page 17 with the following rewritten paragraph:

Figure 3 schematically illustrates a more complex channel geometry for carrying out the concentration methods described herein. In particular, the channel layout ~~300~~ includes sample loading channel segments, e.g., 302a and 302b, that are connected to channel segments 304a and 304b connected to each other by a fluid junction 306 (here shown as channel segment 306). An additional channel segment 302c is provided connected to the fluid junction 306, in order to provide an additional source for the second fluid, e.g., the high conductivity buffer, to provide facilitated set-up of the static interface (see below). In particular, the channel configuration functions substantially as described for Figure 2C, except that the second fluid is provided within channel 302c, as well as in channel segment 304a and 304b. The sample material in channel segment 302a is then subjected to an electric field whereby the sample material is concentrated in the second fluid region in the fluid junction 306. The concentrated material is then directed down channel segment 304b for further manipulation, in the same fashion described above.

Please replace paragraph 0057 on page 17 with the following rewritten paragraph:

In providing an additional high conductivity buffer source channel, e.g., channel 302c, set-up of the static interface is facilitated in the channel network shown in Figure 3A. This set-up is shown schematically in Figure 3B. In particular, as shown, the entire channel network is first filled with the first fluid 310, e.g., low ionic strength, which is indicated by hatching. The second fluid 312 (indicated by cross-hatching) is then simply directed through channel 302b, 304a, 304b and ~~304e~~ 302c. Again, control of flow at the fluid junction is a simple matter of regulating flow in the various channels that are connected at that junction, e.g., by flowing the

second fluid in through channels 304a and ~~304e~~ 302c. A slight level of flow is also optionally applied through channel 302a, in order to prevent movement of the fluid interface 308. Following this set-up, the main static interface 308 will be established at the fluid junction 306. Sample material is then electrophoresed from sample channel 302a (and optionally, 302c) into the fluid junction 306, where it will concentrate just beyond the static interface 308. The concentrated material is then optionally transported into a connected channel segment, e.g., 304b, for additional manipulation or analysis.

Please replace paragraph 0058 beginning on page 17 with the following rewritten paragraph:

Figure 7 illustrates another channel configuration for a microfluidic device suitable for the concentration methods of the present invention for use with a high throughput system. As shown in Figure 7, the device ~~700~~ comprises a fluidic interface such as a pipettor or a capillary 726 in fluid communication with a source of fluid borne sample materials. The sample materials are drawn up through the capillary and into the channel network of the device by applying vacuum on reservoirs 710 and 712 and maintaining the pressure at the remaining reservoirs at atmospheric pressure. In order to carry on an “on-chip” reaction whereby different materials are reacted and analyzed in the microfluidic channel network of the device, reservoir 724 may optionally be used to introduce a reagent or material for interacting with the sample material drawn up through the capillary 726. For example, in an antibody/antigen assay, an antigen may be drawn up in low salt sample fluid via capillary 726 while reservoir 724 is used for introducing the antibody, also contained in a low salt fluid, via channel region 728. The antibody and the antigen form a mixture and incubate in the reaction channel region 730. The flow and residence time within channel region 730 is controlled by simultaneously controlling the pressure at the various reservoirs shown in the device while maintaining a vacuum at reservoirs 712 and 714. Reservoirs 718, 722 and 716 are filled with a high salt gel whereby the flow from these reservoirs fills up channel regions 742, 736 and 746 with the high salt gel. As a result, the reaction mixture is well defined in channel regions 732 and 734 due to pinching created by flow from channel regions 736, 742 and 746 respectively. At this point, the pressure at reservoirs 710 and 712 is set to atmospheric pressure and an electric potential is applied between reservoirs 716 and 718. The

application of the electric potential causes the various species contained in the reaction mixture to flow from a low salt region into a high salt region causing the material to concentrate along the leading static fluid interface 760 shown in Figure 7. The voltage potential is then shifted from being between reservoirs 716 and 718 to between reservoirs 722 and 718 so as to improve the efficiency of the system by performing the separation of the various species in the sample in a substantially homogeneous electric field, i.e., in the substantially homogeneous high salt region which is contained within channel segment 742 without substantial cross-flow contamination from low salt regions 732 and 734. The separated materials flow along the separation channel 742 and past a detection region whereby the highly concentrated and separated material can be easily detected.

Please replace paragraph 0060 on page 19 with the following rewritten paragraph:

Figure 4 schematically illustrates a channel structure useful for carrying out the countercurrent concentration methods of the present invention. Like the static interface methods described above, these countercurrent methods rely upon a shift in velocity of the sample material in one channel segment in order to accomplish the desired concentration. In these methods, however, the velocity shift is due primarily to the counter directional bulk fluid flow, e.g., counter to the direction of electrophoretic movement. As shown in Figure 4A, a main channel 402 is provided, with two side channels 404 and 406 intersecting main channel 402 at two discrete points. The main channel is coupled to a pressure source or other bulk flow system, e.g., electroosmotic pressure pump, pressure or vacuum pump, manifold, etc., or the like. The side channels are each coupled to an electrical power supply, e.g., via electrodes ~~416 and 418~~, for applying an electric field through channels 404 and 406, via channel segment 402a.

Please replace paragraph 0072 on page 22 with the following rewritten paragraph:

Unless otherwise specifically noted, all concentration values provided herein refer to the concentration of a given component as that component was added to a mixture or solution independent of any conversion, dissociation, reaction of that component to alter the component or transform that component into one or more different material once added to the mixture or solution.